

In The United States Patent and Trademark Office

Application number: 10/667,882

Application filed: September 22, 2003

Applicant: Nathaniel Tue Tran

Title: MULTIPLEXING ARRAY TECHNIQUES

APPENDIX A

Marked-up pages showing changes in the Specification and Abstract

BEST AVAILABLE COPY

SUMMARY OF THE INVENTION

Briefly stated, this invention provides a novel means of detection or quantitative comparison of molecules between two samples on arrays of binding agents. The novel method allows competitive bindings of two samples on the same array making quantitative comparison possible. In addition, improved methods uses radioactive labeling offers the highest sensitivity of all available detection methods.

The present invention discloses a method of analysis comprising the steps of: (1) labeling at least one sample of test molecules with a unique labeling agent; (2) mixing the labeled and unlabeled samples of test molecules into a homogenous mixture; (3) applying the mixture to an array of binding agents; (4) washing away any un-reacted or unbound test molecules from the surface of the array; and (5) analyzing the surface for any indication of reaction between resident molecules and test molecules.

A variation of the invention discloses a method of using one readily detectable label (of any kind of labeling agent or distinguishable characteristic of a molecule) as a standard to perform the analysis. The other sample to be multiplexed with may contain a dummy label or no label for the purpose of making similar molecules in both samples behave the same way. For example, naturally occurring carbon-12 would be considered a dummy label for radioactive carbon-14. The method of analysis comprises the steps of: (1) mixing quantities of a standard with two different quantities of samples to produce mixtures having two or more different proportions; (2) applying the different mixtures to identical arrays; (3) washing away unbound molecules; (4) reading the signal from each array; and (5) calculating the amount of various molecules present in the tested sample relative to the amount in the standard sample using the signal variation between various mixtures.

A major advancement in labeling and detection of multiple samples in this invention is the use of radioactive isotopes. Unlike other labeling and bioconjugation techniques, radioactive labeling does not require putting large tags onto biological molecules that often result in significant physical and chemical changes in the properties of the biological

Deleted: 7

Deleted: and

Deleted: fication to enable comparison

Deleted: from

Deleted: or more

Deleted: two

Deleted: s

Deleted: each

Deleted: of samples of test molecules

Deleted: rinsing

Deleted: ing

Deleted: s

Substitute sheet 8

Deleted: 1

The antibodies and binding agents can be arrayed on a microarray slides similar to the microarray slides manufactured by Perkin Elmer. The slides are coated with adsorbance materials or activated surface chemistry to bind or covalently link to binding agents. Of particular use is the method used by Khrapko, et al (1996) for immobilization of oligonucleotides, which can be modified for protein coupling, or Versalinx (Prolinx, Bothell, WA, USA) slides.

Alternatively, one can also couple the antibody through its SH groups to acrylamide that has been modified to include SH groups. The Versalinx slides are pre-modified with salicylthoxamic acid. The phage can be easily modified with phenyl boronic acid and a complex between the phenylboronic acid and salicylthoxamic acid is formed to immobilize the antibody on the glass slide. Alternatively, the slides are coated with resins that impart a positive charge to the slides, which are commercially available. The phage or the bacteria can be arrayed directly on these slides using an automated slide spotter.

Yet another way to immobilize the antibody is to engineer the cDNA that codes for 6-Histidine moieties contiguously with the amino terminal or the carboxyl terminal of the antibody or the Fab fragment. Stomolysin or similar protease sites are engineered on either end of the antibody-Histidine tag fusion protein for easy excision of the antibody such that the antibody or Fab fragment will carry the Histidine tag. The Histidine residues can be utilized to bind the antibody or Fab to slides which have nickel immobilized on their surfaces. Published procedures for immobilizing nickel to glass slides or other supports are available.

The array should now constitute a substrate along with the resident molecules and any molecules derived from the positive and negative samples that have reacted with the resident molecules. The array may now be observed for the presence of the different and unique labels associated with the positive and negative samples, respectively.

Eventually, the array may be stripped of the homogenate mixture and used again or may be disposed, depending on the particular needs of the analyst.

Substitute sheet 14

DESCRIPTION OF THE PREFERRED EMBODIMENTS

With reference to Fig. 1, a first preferred embodiment of the invention demonstrates how differences in level of abundance of many molecules from two samples can be analyzed and compared when one is labeled. The preferred labeling method uses neutron bombardment to add neutrons to existing atoms in test molecules within the samples. The additional neutrons make the molecules heavier or even radioactive. Alternatively, the labels especially radioactive isotopes can be incorporated as natural parts of the molecules in the case of live cells actively synthesizing the molecules of interest. Natural incorporation can also be done by replacement of existing atoms with radioactive isotopes such as replacing hydrogen with tritium by desaturation and resaturation or by proton exchange in tritiated water. Once labeling is completed, the samples are mixed to homogeneity and applied to arrays for analysis. The binding agents on these arrays bind to and profile different types of molecules at distinct locations on the arrays. The radiation from these locations is detected and quantified. When a labeled sample is mixed with unlabeled sample at different proportions and then applied to identical arrays the resulting amount of radiation signal can be used to interpret the relative amount of similar molecules in unlabeled sample relative to those in labeled sample. In addition, when captured molecules are required to be identified by mass spectrometry, the origin of molecules from either sample can also be differentiated by their slight difference in mass. Importantly, the method described provides a way to simultaneously compare the relative abundance of many molecules between two samples and to discover differentially abundant molecules between two samples.

Deleted: :

Deleted: for

Deleted: molecules having varying abundances to be discovered

A second preferred embodiment uses other labeling methods including bioconjugation or covalent modification of molecules to add label or tags onto the molecule of interest. One sample can be tagged with a distinguishable label, tested, and then uses as standard. The standard is then mixed with sample to be tested (unknown) at two or more different proportions and then applied to two identical arrays. The signals from both arrays are read and used to calculate the ratio of unlabeled vs. labeled molecules for every particular spot. Because tagged molecules may behave differently compared to untagged molecules, the tagged molecules needs to be validated before they can be used as standard. This can be

Deleted: in the standard

Deleted: can

Deleted: than

Deleted: in the sample

Deleted: .

Deleted: standard

Deleted: is

Substitute sheet 15

done using similar experimental scheme as described in the first preferred embodiment where neutron bombarded sample will be compared against tagged sample. Both of these samples are of the same origin for the purpose of validating that the bindings of certain molecules have not been affected. Alternatively, tagged and untagged samples originated from the same pool can be used for validation. For instance, equal amount of tagged and untagged samples mixed together should yield half as much signal for all spots on an array compared to just tagged sample alone on an identical array if tagged sample alone can saturate all spots on the array.

While the preferred labels for this embodiment are radioactive isotopes, it is not constraint to just radioactive isotopes. The molecules can be labeled with small tags, such as biotin to be detected with streptavidin or avidin couple to horse radish peroxidase or alkaline phosphatase to generate an enzymatic reaction with detectable end points. Additionally, the molecules can be labeled with big fluorophors if the dye can be incorporated into these molecules in such a way that it won't interfere with the essential properties of the molecules for the particular assays (as determined later on by validation assay). Because the labeling process does not have to be done during the time of analysis, the reagent can be prepared and validated commercially in advance to meet certain standards. If the reagent used as a standard is labeled with non-radioactive tags, then it can be tested and validated first using radioactive labeled molecules as a standard.

A third preferred embodiment covers the use of other molecules besides binding agents on an array. These molecules are spotted on for the purpose of standardizing and comparing across similar arrays. For isotope labeling, same amounts of various isotopes are spotted on the array acting not only as a standard across arrays but also as a calibration tool for calculating screening of a particular type of isotope. For biotinylation labeling, a fixed amount of biotin is spotted onto each array to enable comparison and normalization between two or more arrays.

A fourth preferred embodiment comprises alternative methods to label biological samples without altering their chemical structures or property. Biological samples, such as

Deleted: s

Deleted: are

Deleted: e

Substitute sheet 16

those from humans, can not be readily labeled with radioactive isotopes by metabolic incorporation because such labeling would require ingestion of radioactive materials. Nonetheless, labeled human biological samples are required to enable discoveries of biomarkers in disease condition or drug treatments in human. To overcome the obstacle, this method incorporates neutron activation - the same method used to make most artificially heavy and radioactive isotopes. Letting biological samples be bombarded with neutrons from source such as nuclear reactors or other neutron generating device can turn the samples radioactive over time. Briefly, samples contain stable isotopes such as hydrogen, carbon-12, sulfur-32, phosphorus-31, and few other less abundant trace elements that can gain additional neutrons and become radioactive isotopes such as tritium, Carbon-14, Sulfur-35, Phosphorus-32 or Phosphorus-33 as a result of neutron bombardment. In addition other stable isotopes are also formed as a result of neutron bombardment. Isotopes such as ^2H , ^{13}C , ^{15}N , and ^{18}O can code these samples to be differentiated from regular isotopes by mass spectrometry.

Formatted: Superscript

Formatted: Font: (Default) Times New Roman

The above descriptions provide methodologies for which a broad spectrum of particular analysis may apply. Accordingly, the following examples are provided to further enhance the users understanding of the invention but in no way are intended to limit the particular application of the method described above.

EXAMPLES

Deleted: ¶
¶

Example 1: High-throughput drug screening assay development

Formatted: Underline

Formatted: Left

Formatted: Underline

A cell line is used to study the effect of a drug and also to develop new high-throughput screenings for new drugs. Cells are grown in identical ways in various culture flasks at the beginning before separating into different groups. One group is treated with a known drug (with known therapeutic efficacy) while the cell are metabolically labeled with ^{14}C -Cystein and Methionine. Another group is treated with vehicle control (the same amount of solvents used to deliver the drug) while the cells are metabolically labeled with ^3H -Cystein and Methionine. Other groups are treated with potential compounds while being labeled with ^{35}S -Cystein and Methionine. Samples from ^{14}C and ^3H labeled cells are mixed together and

Substitute sheet 17

apply to protein arrays to look for changes in protein expression. The proteins from both samples compete for binding to binding agents on the array. As a result, if more of a particular protein is present in one sample, then more of that protein would also bind to its specific binding agent in the exact proportion. The array is then washed with different stringency buffers to retain only certain bindings and then read for radioactivity.

Detection: Tritium emits weak beta radiation that more than 95% can be blocked by a thin layer of saran wrap. Carbon-14 and sulfur-35 emit stronger radiation that approximately 50% pass through the saran wrap. Using this principle, reading all of the signal from the array and then reading the signal screened by a thin film such as the saran wrap will enable one skilled in the art to calculate how much signal belongs to tritium and how much belongs to ^{14}C and ^{35}S . In addition, radiation from individual isotopes can also be quantified with all three isotopes mixed together. This is accomplished by first reading the total signal, then reading the signal with a tritium screen. The array may be stored for a period of time before reading again. If the storage period is 87.4 days (one half-life of ^{35}S), then when it is read again, the reduction in total signal is equal to half the signal from ^{35}S . Simple mathematical calculations will quantify the signal from ^{14}C and tritium. In addition, the arrays that are destined for these types of experiments may contain spots of these radioactive isotopes on them to enable better calibration. Specialized software, also known to one skilled in the art, ultimately can perform all of the calculations necessary to separate the combined signals between different isotopes.

After reading signals from each isotope, these signals are compared, for their respective increase or decrease in the different proteins' expressions, and new drug treatments are pinpointed. Spots on the array that show significant changes are used as biomarkers for further drug discovery. Together, these spots form a "profiling map" that one would look for in test compounds. In addition, the protein from any spot can be identified if the binding agent at that spot is known to capture a specific protein. If not, then the same antibody can be used to capture a large amount of that particular protein by methods such as immunoprecipitation, and then identify the captured protein using mass spectrometry.

Deleted: completely

Substitute sheet 18

Using the "profiling map", one can look for similar changes in compounds being tested. Compounds that show enough similarity can be further studied for their comparable therapeutic effect. In addition, knowing the biomarkers that change as a result of drug treatments can provide insights into how a particular drug works or what possible drug-targets there are. All of this knowledge can lead to better ways in the development and screening of new drugs.

Example 2: Quantitative comparison by array analysis using only one labeled sample

Whenever it is not possible to label both samples with different radioactive isotopes, only one sample needed to be labeled for used with slight modification to the above procedure. A known amount of labeled sample is first used to determine a necessary minimum amount needed to saturate every spot on the array of interest. This is achieved when further increasing in the amount of sample used won't increase the amount of signal read at any dots on these arrays. This amount is used to establish a reference to compare reading from other arrays. Then the assays are performed by mixing approximately equal amount of labeled sample and unlabeled sample together for competitive binding on the same array. The signals read on this array will be compared against reference signals for quantitative analysis. Some spots on the arrays are used to capture housekeeping proteins. The signal reduction on these spots will be used as reference standards for comparison. For instance, if the mixture is exactly equal amount of proteins between labeled and unlabeled samples then the reference signal read should be reduced by half. As a result, any other spots with signals reduced by more than half (or the percentage reduction observed with housekeeping proteins) have more unlabeled antigen than labeled antigens and vice versa.

This method is especially useful for clinical samples when one sample can be pre-labeled and used as comparison standard. It is also useful in combination with our novel labeling technique using neutron bombardment to randomly making various isotopes within the sample radioactive. Pre-labeling also allows time for validation to ensure that the labeling process does not change qualities of molecules being labeled and making them

Deleted: 1

Formatted: Underline

Formatted: Underline

Formatted: Left

Deleted: Post-translational modifications play an important role in protein functions. Protein can be modified by the addition of phosphates, sugars, lipids, or steroids, methyl groups, nitrate groups, etc. These modifications may be studied using the same approach described in this invention for labeling samples with different isotopes and then profiling on an array.

Phosphorylation study

Two sets of cells are plated and treated identically from the beginning, then one set is treated with drug while being labeled with ^{32}P phosphates, and the other set is treated with a vehicle while being labeled with ^{32}P phosphates. After a defined treatment time, the labeling media is washed away. The cells are next harvested and mixed together for analysis. Protein samples can be prepared for profiling on protein arrays while RNA samples can be prepared for profiling on gene arrays (a long labeling time will also label RNA and DNA). Protein-RNA and protein-DNA interactions can also be studied in addition to just protein-protein interactions.

Substitute sheet 19

unsuitable for competitive binding with unlabeled molecules to a binding agent in an array of interest

Deleted: ¶

5 Example 3: Ultra-sensitive detection of antigen

Formatted: Underline

Formatted: Left

B. anthracis is grown in medium with radioactive precursors and induced to make antigens normally present in human infection such as protective antigens, lethal factors, edema factors. The specific radioactive proteins are purified to be used as standards.

- 10 Antibodies are also made against these antigens to use in diagnosing early anthrax exposure. Antibodies are immobilized on beads or array for the purpose of the assays.

The amount of standard used will always contain more antigens than available antibodies on the array. Thus this standard alone or in combination with other sample will always saturate the binding capacity of the array. The radiation signal is read when the standard is used alone and as a mixture with healthy samples. Both of these readings should be approximately the same to make reliable diagnosis. The mixtures of standard and samples from anthrax exposed animals are used to determine if there are any changes in radiation reading. A significant change in the radiation reading means positive detection of the same antigens used in the standard that are captured by antibodies on the array. Different mixtures with samples taken at different stages of exposure will determine how early the assay can be used to detect anthrax exposure.

Deleted: y

Deleted: and

25 Example 4: Differential proteomics for clinical diagnostic application

Formatted: Underline

Formatted: Left

- 30 Normal human clinical samples are taken and frozen for preservation. These samples are then bombarded with neutrons for neutron activation. When the samples have become sufficiently radioactive, they can be used as standards to look for changes in other samples by using the steps below as a guide:

Substitute sheet 20

1. Use a fixed amount of standard on the array to obtain standard only signal. The amount used must be sufficient to saturate every spot on the array. This is determined when increasing the amount used won't increase the signal read any further.
2. Mix the fixed amount of standard with approximately the same amount of sample to be tested. Determine the percentage of signal reduction at spots that capture housekeeping proteins such as β -actin.
3. Use the percentage of signal reduction in other spots to determine the relative amount of proteins between samples and standard. Housekeeping proteins represent equal amount between sample and standard. If signal reduction percentage is higher at a spot (compared to percentage reduction of housekeeping proteins) then more proteins from sample than standard is present at that spot (and vice-versa). Therefore that particular protein is more abundance in the sample compared to the standard.
4. An additional mixture can be used with slightly different mixing ratio if necessary to increase the reliability of the analysis. Such additional steps would be required when validating the assay. If using 1:1 of standard : sample mixing ratio resulting in 50% signal reduction, then 1:2 mixture would result in ~66.7% signal reduction and so on.

Using samples from normal/healthy donors bombarded with neutrons to make standards, one can look for changes of biomarkers in diseased or drug-treated patients. These biomarkers can later be used to diagnose the diseases or test drugs' efficacies such as screening for new potential drugs with similar therapeutic efficacies.

In addition to making the sample radioactive by neutron bombardment, other known methods such as tritiated water exchange can also be used. Briefly the molecules to be labeled are dialyzed in tritiated water at high pH where ionizable protons are exchanged with ^3H protons from water. Then when the pH is lowered to slow down or stop such exchange when these labeled molecules are used for an experiment. This tritiated water exchange method has been well-known and frequently practiced by those skilled-in-the art as a means for labeling biological molecules with tritium.

Example 5: Differential proteomics without radioactive labeling

Substitute sheet 21

Formatted: Indent: Left: 0"

Formatted: Indent: Left: 0", First line: 0.5"

Formatted: Indent: Left: 0"

Formatted: Indent: Left: 0", First line: 0.5"

Formatted: Underline

Formatted: Left

Similar to example 4, this method uses non-radioactively labeled sample such as biotin labeled sample as standard. Because such labeling necessitate the addition or chemical modification of existing molecules, the standard needs to be validated to make sure that such modification is compatible with the array used. For such validation, competitive binding with the same sample without any label is done where unlabeled portion of a sample should complete equally with labeled portion of the same sample. Clinical samples are tagged with biotin or other non-radioactive labels so that they can be readily detectable and quantifiable. The tagged samples are used to compete with the identical samples that have been neutron activated. Any spots with consistent signal reduction indicated that the biotin tags have not interfered with protein binding to those spots. Those are the spots that can be used with the biotin tagged standards. Once validated, an array containing only good working spots plus some spots with fixed amount of immobilized biotin (or other labels) can be used for quantitative analysis. Additionally, when performing multiplexing with neutron activated samples, non-radioactive labels are quantified to establish references.

Deleted: 5

Deleted: neutron activated samples is done

Example 6: DNA array method

Cells are grown in ^{32}P or ^{33}P phosphates while undergoing different treatments. Any resulting difference in RNA expression as a result of these treatments is then examined. After labeling, the cells are collected and approximately equal amounts from ^{32}P and ^{33}P labeling are mixed together for comparative analysis. RNA is extracted, and DNA is digested away using RNase-free DNase. The RNA is then bound to a DNA array for profiling and comparison. The resulting individual signal from ^{32}P vs. ^{33}P can then be calculated by selective screening or by pre-decay and post-decay comparison. Briefly, detecting total signal and partial signal blocked by an X-ray film to quantify signal from each isotope based on the different percentage of radiation from each isotope passing through the film. By decaying for a period of time and match the decaying amount to the right ratio mixture of isotopes to quantify the amount of each isotope. This type of labeling and detection allows much higher degree of sensitivity thus only very small amount of samples

Formatted: Underline

Formatted: Left

Formatted: Underline

Substitute sheet 22

are required for the analysis. Furthermore, the improved sensitivity also enables detection of extremely low abundance RNA without further amplification.

5 Similar to example 2, one can also use one set of labeled RNA to make quantitative comparison of multiple unique RNA molecules with an unlabeled sample. Such methodologies are useful especially when labeled RNA from normal cells or tissue can be conveniently obtained through commercial sources for comparative analysis with treated cells. For instance, a commercial source can supply labeled reference standards of proteins, RNA and other biomolecules of interest from cells or tissues in their normal healthy states.
10 The investigators can use these labeled standards for multiplexing analysis with their diseased or drug-treated samples to look for any resulting changes. When the changes are known to be associated with a disease or drug treatment, then the method can be used for rapid disease diagnosis or high-throughput drug screening.

15 Example 7: Point-of-care rapid medical diagnostic system

20 Incorporating methods described in previous examples, patient samples can be compared with healthy samples preferably from the same patient taken when healthy to make reliable diagnosis. Blood plasma samples from a patient are taken during routine checkup when the patient is healthy. This sample is labeled preferably with long-live radioactive isotopes such as ^3H or ^{14}C or non-radioactive tags such as biotin or fluorescent dyes. Labeled samples and unlabeled samples from the same pool are validated on diagnostic arrays to ensure that the labeling procedures do not affect competitive binding to these arrays. The
25 validation procedure also provides useful reference information such as signal read for saturated array and half-saturated array. These labeled samples can be store frozen in aliquots for future use.

30 To make diagnostic testing, blood plasma sample from the patient is taken, mixed with his labeled samples and profiled on the same antibody array. Signals read from this competitive binding are then compared with reference signals read during validation

Formatted: Underline

Formatted: Left

Formatted: Underline

Deleted: When labeling methods discussed herein can not be incorporated due to some limit on practicality or sensitivity of a biological molecule, often, DNA or RNA interaction with proteins can be substituted as a label.

Accordingly, even when an analysis is not possible with other labeling methods, the processes between DNA, RNA, and protein may provide a sufficient distinguishing characteristic to identify a molecule after mixing. This is of particular advantage because interactions between proteins, DNA, RNA, lipids... etc. are very important in cellular metabolism. Such interactions offer further uses in drug targeting. ¶

¶ In this example, the term "label" includes a distinguishing characteristic and does not require the inclusion of a chemical tag, biomarker, or labeling agent. ¶

¶ To assist in separating DNA and RNA samples, agarose beads may be used to immobilize one type of molecule in and an array can be used to capture the other type of molecule.

Substitute sheet 23

procedure. The results should yield knowledge of any increase or decrease in a particular antigen abundance. Such information is matched to known diseased-associated changes to provide a diagnosis.

Formatted: Left

Example 8: Comparing DNA methylation

Formatted: Underline

Formatted: Left

DNA methylation is well known as a distinguishing DNA characteristic, particularly when concerning diseases, such as cancers or other regulatory mechanisms such as genetic imprinting. Determine the extent of DNA methylation and which genes have been methylated can facilitate the diagnosis and subtyping of cancers.

For DNA methylation analysis, cells samples are collected from patients to be used for comparison with a normal or healthy donor's sample. DNA is extracted, and then methylated with either tritium or ^{14}C labeled methyl donor compounds such as S-[methyl- ^{14}C]-Adenosyl-L-Methionine, or S-[methyl- ^3H]-Adenosyl-L-Methionine. Then equal amount of DNA from both normal control and patient's sample are mixed together for restriction enzyme digestion. The digested fragments are applied to DNA arrays and the signal from tritium and ^{14}C detected and quantified.

Deleted: or

Deleted: S-[methyl- ^{14}C]

The logic here is that any methylation site on the DNA that has already been methylated won't be methylated again with radioactively labeled methyl groups. As a result, the more DNA has been methylated, the fewer labels it will acquire in the labeling process.

Deleted: reasoning

Deleted: mixing

In addition, multiplexing patient DNA against normal donor DNA or his own DNA collected previously allows identification of genes that are abnormally methylated. These genes can be used as biomarkers to diagnose or better understand the disease in the future. When the methylations of such genes are well characterized, it will be possible to only use the patient's samples alone, along with the patient's history, on a DNA array to determine the methylation of any particular gene. These genes are of special interest in contributing to the disease and may be used to devise diagnoses or treatments

Substitute sheet 24

One type of label can also be used to determine if there are any changes in DNA methylation pattern. Patient DNA is mixed with fixed amount of exogenous DNA before subject to methylation with isotope donors. Exogenous DNA has known amount of methylation sites and degree of methylation thus is used to control for methylation reaction efficiency. After methylation, the DNA mixture is digested by restriction enzymes and the resulting DNA fragments profiled on a DNA array. There should be sufficient quantity of DNA to saturate all spots on the array for the analysis to work. The signals from spots where exogenous DNA is captured are used as reference standard to make comparison between arrays. Patterns of signals recorded from one array analysis can be used to compare to signals in another array analysis to look for aberrations.

Importantly, while this example describes using a radioactive methyl group to perform the experiment, those skilled in the art can also use variation with non-radioactive labels to modify DNA and perform the study.

The examples herein should not be interpreted as an exhaustive or comprehensive list of the possible applications of the present invention. The present invention is a method for combining labeled and unlabeled molecules, exposing them to an array, and finally observing and quantifying the amount of labels to interpret relative abundance of molecules between samples. Analysis and observations may be automated to such an extent that clinical diagnosis is entirely instrumental and automated. In fact, from sample collection, mixing, array application, and analysis may be completely automated and not require any manual handling or processing.

Having disclosed my invention in such terms as to enable those skilled in the art to understand and practice it, and having identified the presently preferred embodiments thereof, I CLAIM:

Deleted:

Deleted: distinguishable

Deleted: a characteristic of the distinguishable molecule after array.

Formatted: Font color: Auto

Substitute sheet 25

ABSTRACT

5 A novel method for labeling, detecting, comparing and quantifying molecules from
two samples on the same array is described. The method uses at least one labeled sample to
be mixed together with one other sample and then allows competitive binding to an array of
binding agents. The array captures and profiles various molecules from both samples at
predetermined locations for detection and quantitative comparison. When mixing labeled
and unlabeled samples at different proportions, relative ratio of labeled and unlabeled
molecules can be identified. Such relative ratio in combination with a reference ratio can be
10 used to identify if a certain molecule is more or less abundant between the two samples. The
preferred label used for this invention is radioactive isotope. When only labeling one sample,
the sample can be labeled using neutron bombardment without changing the chemical
structures of molecules within. Alternatively, both samples can be labeled with chemically
identical groups containing different radioactive isotopes and then utilizing the isotopes
15 differences in half-life or radiation energy to selectively quantify each of them from the
mixture. This method can be used for biomarkers discovery and rapid profiling of such
biomarkers to enable point-of-care diagnostic testing and personalized medicine. The
method also has applications in proteomics, phosphor-proteomics, transcriptomics,
metabolomics, genomics, and glycomics studies.

Deleted: multiple

Deleted: an unlabeled

Deleted: By

Deleted: The signals detected for various molecules from labeled sample will provide indication of presence and relative amount of the same molecules in the unlabeled sample.

Substitute sheet 31

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- | | |
|--|--|
| <input type="checkbox"/> BLACK BORDERS | <input type="checkbox"/> BLACK BORDERS |
| <input type="checkbox"/> IMAGE CUT OFF AT TOP, BOTTOM OR SIDES | <input type="checkbox"/> IMAGE CUT OFF AT TOP, BOTTOM OR SIDES |
| <input type="checkbox"/> FADED TEXT OR DRAWING | <input type="checkbox"/> FADED TEXT OR DRAWING |
| <input type="checkbox"/> BLURRED OR ILLEGIBLE TEXT OR DRAWING | <input type="checkbox"/> BLURRED OR ILLEGIBLE TEXT OR DRAWING |
| <input type="checkbox"/> SKEWED/SLANTED IMAGES | <input type="checkbox"/> SKEWED/SLANTED IMAGES |
| <input type="checkbox"/> COLOR OR BLACK AND WHITE PHOTOGRAPHS | <input type="checkbox"/> COLOR OR BLACK AND WHITE PHOTOGRAPHS |
| <input type="checkbox"/> GRAY SCALE DOCUMENTS | <input type="checkbox"/> GRAY SCALE DOCUMENTS |
| <input type="checkbox"/> LINES OR MARKS ON ORIGINAL DOCUMENT | <input type="checkbox"/> LINES OR MARKS ON ORIGINAL DOCUMENT |
| <input type="checkbox"/> REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY | <input type="checkbox"/> REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY |
| <input type="checkbox"/> OTHER: _____ | <input type="checkbox"/> OTHER: _____ |

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.